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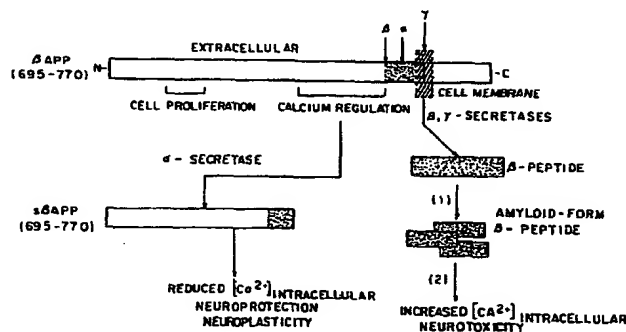
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(54) Title: CHIMERIC PEPTIDES AS IMMUNOGENS, ANTIBODIES THERETO, AND METHODS FOR IMMUNIZATION USING CHIMERIC PEPTIDES OR ANTIBODIES

(57) Abstract: The invention provides a chimeric peptide or mixture of chimeric peptides that can be formulated as an immunizing composition and used in a method for immunization of a mammal against an internal peptide cleavage product derived from a precursor or mature protein, for which the peptide cleavage product and the precursor or mature protein are self molecules. The chimeric peptide or peptides have an end-specific B cell epitope from a naturally-occurring internal peptide cleavage product of a precursor or mature protein, as a free N- or C- terminus, fused with or without spacer residues to a T helper cell epitope derived from a living source different from that of the internal peptide cleavage product.

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CHIMERIC PEPTIDES AS IMMUNOGENS, ANTIBODIES THERETO, AND METHODS FOR IMMUNIZATION USING CHIMERIC PEPTIDES OR ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

5   The present application claims the benefit of priority from U.S. provisional application no. 60/169,687, filed December 8, 1999, the entire contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

10   The present invention relates to a chimeric peptide immunogen containing a B cell epitope joined to a T cell epitope from a different source, immunizing compositions containing the chimeric peptide, and a method for immunization using same.

Description of the Background Art

15   A major histopathological hallmark of Alzheimer's Disease (AD) is the presence of amyloid deposits within neuritic and diffuse plaques in the parenchyma of the amygdala, hippocampus and neocortex (Glenner and Wong, 1984; Masters et al., 1985; Sisodia and Price, 1995). Amyloid is a generic term
20 that describes fibrillar aggregates that have a common β-pleated structure. These aggregates exhibit birefringent properties in the presence of Congo red and polarized light (Glenner and Wong, 1984). The diffuse plaque is thought to be relatively benign in contrast to the neuritic plaque which appears to be strongly
25 correlated with reactive and degenerative processes (Dickson et al., 1988; Tagliavini et al., 1988; Yamaguchi et al., 1989; Yamaguchi et al., 1992). The principal component of neuritic plaques is a 42 amino acid residue amyloid-β (Aβ) peptide (Miller et al., 1993; Roher et al., 1993) that is derived from
30 the much larger β-amyloid precursor protein, βAPP (or APP) (Kang et al., 1987). Two major C-terminal variants of amyloid-β peptide, Aβ 1-40 ending at Val40 and Aβ 1-42(43) ending at Ala42 or Thr43, proteolytically cleaved from βAPP, were found in amyloid deposits (Miller et al., 1993; Roher et al., 1993). Aβ

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1-42 is produced less abundantly than the 1-40 Aβ peptide (Haass et al., 1992; Seubert et al., 1992), but the preferential deposition of Aβ1-42 results from the fact that this COOH-extended form is more insoluble than 1-40 Aβ and is more prone
5 to aggregate and form anti-parallel β-pleated sheets (Joachim et al., 1989; Halverson et al., 1990; Barrow et al., 1992; Burdick et al., 1992; Fabian et al., 1994). Aβ1-42 can seed the aggregation of Aβ 1-40 (Jarrett and Lansbury 1993). Iwatsubo et al., (1996) and Saido et al., (1996) further reported that other
10 variant amyloid-β peptides, Aβ 3(pyroglutamate)-42, Aβ 11(pyroglutamate)-42, Aβ 17-42, Aβ 1(D-Asp)-42, and Aβ 1(L-isoAsp)-42 were also found to be present in amyloid deposits in the brain.

The APP gene was sequenced and found to be encoded on
15 chromosome 21 (Kang et al., 1987). Expression of the APP gene generates several Aβ-containing isoforms of 695, 751 and 770 amino acids (Figure 1), with the latter two βAPP containing a domain that shares structural and functional homologies with Kunitz serine protease inhibitors (Kang et al., 1987; Kitaguchi
20 et al., 1988; Ponte et al., 1988; Tanzi et al., 1988; Konig et al., 1992). The functions of βAPP in the nervous system remain to be defined, although there is increasing evidence that βAPP has a role in mediating adhesion and growth of neurons (Schubert et al., 1989; Saitoh et al., 1994; Saitoh and Roch, 1995) and
25 possibly in a G protein-linked signal transduction pathway (Nishimoto et al., 1993). In cultured cells, βAPPs mature through the constitutive secretory pathway (Weidemann et al., 1989; Haass et al., 1992; Sisodia 1992) and some cell-surface-bound βAPPs are cleaved within the Aβ domain by an enzyme,
30 designated α-secretase, (Esch et al., 1990), an event that precludes Aβ amyloidogenesis (Figure 1). Several studies have delineated two additional pathways of βAPP processing that are both amyloidogenic: first an endosomal/lysosomal pathway generates a complex set of βAPP-related membrane-bound
35 fragments, some of which contain the entire Aβ sequence (Haass et al., 1992; Golde et al., 1992); and second, by mechanisms that are not fully understood, Aβ 1-40 is secreted into the

conditioned medium and is present in cerebrospinal fluid *in vivo*
(Haass et al., 1992; Seubert et al., 1992; Shoji et al., 1992;
Busciglio et al., 1993). Lysosomal degradation is no longer
thought to contribute significantly to the production of $A\beta$
5 (Sisodia and Price, 1995). The proteolytic enzymes responsible
for the cleavages at the NH_2 and COOH termini of $A\beta$ termed β and
γ (Figure 1), respectively, have not been identified. Until
recently, it was generally believed that $A\beta$ is generated by
aberrant metabolism of the precursor. The presence, however, of
10 $A\beta$ in conditioned medium of a wide variety of cells in culture
and in human cerebrospinal fluid indicate that $A\beta$ is produced as
a normal function of cells.

If amyloid deposition is a rate-limiting factor to
produce AD, then all factors linked to the disease will either
15 promote amyloid deposition or enhance the pathology that is
provoked by amyloid. The likelihood of amyloid deposition is
enhanced by trisonomy 21 (Down's syndrome) (Neve et al., 1988;
Rumble et al., 1989), where there is an extra copy of the APP
gene, by increased expression of APP, and by familial
20 Alzheimer's Disease (FAD)-linked mutations (Van Broeckhoven et
al., 1987; Chartier-Harlin et al., 1991; Goate et al., 1989;
Goate et al., 1991; Murrell et al., 1991; Pericak-Vance et al.,
1991; Schellenberg et al., 1992; Tanzi et al., 1992; Hendricks
et al., 1992; Mullan et al., 1992). Some of these mutations are
25 correlated with an increased total production of $A\beta$ (Cai et al.,
1993; Citron et al., 1992) or specific overproduction of the
more fibrillogenic peptides (Wisniewski et al., 1991; Clements
et al., 1993; Suzuki et al., 1994) or increased expression of
factors that induce fibril formation (Ma et al., 1994;
30 Wisniewski et al., 1994). Collectively, these findings
strongly favor the hypothesis that amyloid deposition is a
critical element in the development of AD (Hardy 1992), but of
course they do not preclude the possibility that other age-
related changes associated with the disease, such as paired
35 helical filaments, may develop in parallel rather than as a
result of amyloid deposition and contribute to dementia
independently.

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The main focus of researchers and the principal aim of
those associated with drug development for AD is to reduce the
amount of Aβ deposits in the central nervous system (CNS).
These activities fall into two general areas: factors affecting
5 the production of Aβ, and factors affecting the formation of
insoluble Aβ fibrils. A third therapeutic goal is to reduce
inflammatory responses evoked by Aβ neurotoxicity.

With regards to the first, a major effort is underway
to obtain a detailed understanding of how newly synthesized βAPP
10 is processed for insertion into the plasma membrane and to
identify the putative amyloidogenic secretases that have been
assigned on the basis of sites for cleavage in the mature
protein. From a pharmacological perspective, the most direct
way of reducing the production of Aβ is through direct
15 inhibition of either β or γ secretase. No specific inhibitors
are currently available although a number of compounds have been
shown to indirectly inhibit the activities. Bafilomycin, for
example, inhibits Aβ production with an EC_{50} of about 50 nM
(Knops et al., 1995; Haass et al., 1995), most likely through
20 its action as an inhibitor of vascular $H^{+}ATPase$ co-localized in
vesicles with the Aβ secretase. Another compound, MDL28170,
used at high concentrations appears to block the activity of γ
secretase Higaki et al., 1995). It is generally hoped that the
identification of the β or γ secretases might lead to the
25 synthesis of specific protease inhibitors to block the formation
of amyloidogenic peptides. It is not known, however, whether
these enzymes are specific for βAPP or whether they perhaps have
other important secretory functions.

Similarly, problems of target and targeting
30 specificity will be encountered through any attempt to interfere
with signal transduction pathways that may determine whether
processing of βAPP is directed through the amyloidogenic or non-
amyloidogenic pathways. Moreover, these signal transduction
mechanisms still need to be identified. In conclusion, present
35 understanding of the complex and varied underlying molecular
mechanisms leading to overproduction of Aβ offers little hope
for selective targeting by pharmacological agents.

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Given that neurotoxicity appears to be associated with
β-pleated aggregates of Aβ, one therapeutic approach is to
inhibit or retard Aβ aggregation. The advantage of this
approach is that the intracellular mechanisms triggering the
5 overproduction of Aβ or the effects induced intracellularly by
Aβ need not be well understood. Various agents that bind to Aβ
are capable of inhibiting Aβ neurotoxicity in vitro, for
example, the Aβ-binding dye, Congo Red, completely inhibits Aβ-
induced toxicity in cultured neurons (Yankner et al., 1995).
10 Similarly, the antibiotic rifampacin also prevents Aβ
aggregation and subsequent neurotoxicity (Tomiyama et al.,
1994). Other compounds are under development as inhibitors of
this process either by binding Aβ directly, e.g., hexadecyl-N-
methylpiperidinium (HMP) bromide (Wood et al., 1996), or by
15 preventing the interaction of Aβ with other molecules that
contribute to the formation of Aβ deposition. An example of
such a molecule is heparan sulfate or the heparan sulfate
proteoglycan, perlecan, which has been identified in all
amyloids and is implicated in the earliest stages of
20 inflammation associated amyloid induction.

Heparan sulfate interacts with the Aβ peptide and
imparts characteristic secondary and tertiary amyloid structural
features. Recently, small molecule anionic sulfates have been
shown to interfere with this reaction to prevent or arrest
25 amyloidogenesis (Kisilevsky, 1995), although it is not evident
whether these compounds will enter the CNS. A peptide based on
the sequence of the perlecan-binding domain appears to inhibit
the interaction between Aβ and perlecan, and the ability of Aβ-
derived peptides to inhibit self-polymerization is being
30 explored as a potential lead to developing therapeutic
treatments for AD. The effectiveness of these compounds in
vitro, however, is likely to be modest for a number of reasons,
most notably the need for chronic penetration of the blood brain
barrier.

35   As another means of inhibiting or retarding Aβ
aggregation, WO 96/25435 discloses the potential for using a
monoclonal antibody, which is end-specific for the free C-

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terminus of the Aβ 1-42 peptide, but not for the Aβ 1-43
peptide, in preventing the aggregation of Aβ 1-42. While the
administration of such an Aβ end-specific monoclonal antibody is
further disclosed to interact with the free C-terminal residue

5 of Aβ 1-42, thereby interfering with and disrupting aggregation
that may be pathogenic in AD, there is no specific disclosure on
how these Aβ C-terminal-specific monoclonal antibodies would be
used therapeutically. Although direct or indirect manipulation
of Aβ peptide aggregation appears to be an attractive

10 therapeutic strategy, a possible disadvantage of this general
approach may be that pharmacological compounds of this class
will need to be administered over a long period of time, and may
accumulate and become highly toxic in the brain tissue.

WO 98/44955 takes a novel approach to avoiding the
15 problems associated with repeated administration of
pharmacological agent and discloses a method for preventing the
onset of Alzheimer's Disease or for inhibiting progression of
Alzheimer's Disease through the stable ectopic expression in the
brain of recombinant antibodies end-specific for amyloid-β

20 peptides.

Recently, Schenk et al. (1999) demonstrated that
immunization with amyloid-β attenuated Alzheimer's disease-like
pathology in PDAPP transgenic mice serving as an animal model
for amyloid-β deposition and Alzheimer's disease-like

25 neuropathologies. They reported that immunization of young
animals prior to the onset of Alzheimer's disease-type
neuropathologies essentially prevented the development of β-
amyloid plaque formation, neuritic dystrophy and astragliosis,
whereas treatment in older animals after the onset of

30 Alzheimer's disease-type neuropathologies was observed to reduce
the extent and progression of these neuropathologies.

Although the results reported by Schenk et al.
provides promise for using immunomodulation as a general
approach to treat Alzheimer's disease, immunization with intact

35 amyloid-β according to Schenk et al. has several problems that
need to be addressed in developing an immunization program for
treatment of Alzheimer's disease in humans. One problem is that

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it is not clear how readily one can raise an anti-self antibody
response by immunizing humans with human amyloid-β. Moreover,
even if an anti-self antibody response is raised against human
amyloid-β, it is unclear whether or not auto-immunity might
5 develop which would be injurious to the patient. Other problems
include how the immunization would be reversed or halted as the
antigen in the form of endogenous amyloid-β is always available
to the patients' immune system and whether or not administering
amyloid-β, which is believed to be neurotoxic, would have severe
10 and adverse pharmacological effects on the patient.

        An alternative to a peptide-based approach is to
elucidate the cellular mechanism of Aβ neurotoxicity and develop
therapeutics aimed at those cellular targets. The focus has
been on controlling calcium dysfunction of free radical mediated
15 neuronal damage. It has been postulated that Aβ binds to RAGE
(the receptor for advanced glycation end-products) on the cell
surface, thereby triggering reactions that could generate
cytotoxic oxidizing stimuli (Yan et al., 1996). Blocking access
of Aβ to the cell surface binding site(s) might retard
20 progression of neuronal damage in AD. To date there are no
specific pharmacological agents for blocking Aβ-induced
neurotoxicity.

        Citation of any document herein is not intended as an
admission that such document is pertinent prior art, or
25 considered material to the patentability of any claim of the
present application. Any statement as to content or a date of
any document is based on the information available to applicant
at the time of filing and does not constitute an admission as to
the correctness of such a statement.

30 **SUMMARY OF THE INVENTION**

        The present invention provides a chimeric peptide or
mixture of chimeric peptides with an end-specific B cell epitope
from a naturally-occurring internal peptide cleavage product of
a precursor or mature protein, as free N-terminus or C-terminus,
35 fused with or without spacer amino acid residue(s) to a T helper

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cell epitope derived from a source different from that of the
internal peptide cleavage product.

The present invention also provides an immunizing
composition which includes an immunizing effective amount of the
5 chimeric peptide or mixture of chimeric peptides. This
immunizing composition is used to raise antibodies in a subject
mammal where the internal peptide cleavage product, for which
the B cell epitope of the chimeric peptide is end-specific, is a
self molecule.

10   Further provided by the present invention is a method
for immunization of a mammal against the free N-terminus or free
C-terminus of an internal self peptide cleavage product derived
from a self precursor or mature protein. More particularly, the
method for immunization is directed to raising antibodies and
15 immunizing individuals against amyloid β peptides associated
with amyloid β deposits and plaques.

Yet other aspects of the present invention are
directed to a molecule which includes the antigen-binding
portion of an antibody specific for the chimeric peptide
20 according to the present invention and a method for passive
immunization using this molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic representation of the β-
amyloid precursor protein (βAPP) and the products of α, β, γ-
25 secretase cleavage. The general locations of various domains
are indicated along with the cleavage sites for α, β, and γ-
secretases.

Figure 2 shows a partial amino acid sequence (SEQ ID
NO:1) of the region of βAPP from which amyloid-β peptides (Aβ)
30 are derived. The arrows indicate the α-, β-, and γ- secretase
cleavage sites.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention is directed
generally to a chimeric peptide or a mixture of chimeric
35 peptides in which a N- or C-terminal end-specific B cell epitope

from a naturally-occurring internal peptide cleavage product of
a precursor or mature protein, as a free N- or C-terminus, is
fused with or without spacer amino acid residue(s) to a T helper
cell epitope from a different source. The chimeric peptide or
5 peptides are used in an immunizing composition for immunizing a
mammal against the free N-terminus or free C-terminus of an
internal peptide cleavage product which is a self molecule of
the immunized mammal (molecule native thereto). More
specifically as a preferred embodiment of the present invention,
10 the chimeric peptide(s) have an N- or C-terminal end-specific B
cell epitope, which is the first two to five amino acid residues
of the N-terminus or the last two to five amino acid residues of
the C-terminus of an amyloid β peptide, fused to a T helper cell
epitope. When such chimeric peptide(s) are administered to a
15 human individual as part of an immunizing composition, that
individual will be immunized against the amyloid β peptide or
peptides from which the end-specific B cell epitope is derived.

By way of the preferred embodiments of a chimeric
peptide containing an amyloid β peptide-specific B cell epitope
20 and a method for immunizing against an amyloid β peptide as
examples of the present invention, those of skill in the art
will readily appreciate that the chimeric peptides(s),
immunizing composition, and method for immunization can be
extended and directed to other internal peptide cleavage
25 products, for which end-specific antibodies raised by the
present method would not be cross-reactive with the precursor or
mature self protein. Similarly, most of the advantages
described below for the method for immunization according to the
present invention, which raises end-specific antibodies against
30 amyloid β peptides in humans as a preferred embodiment, are
generally applicable for immunization with the chimeric peptide
or peptides of the present invention.

It is well-known that antibody responses produced by B
cells to a defined region of a protein or peptide require that T
35 helper cells of the immune system recognize another part of that
antigen simultaneously. This is commonly referred to as B/T
cell collaboration. According to the present invention, this

phenomena can be mimicked by making a synthetic chimeric peptide which contains both B and T cell epitopes in a contiguous linear sequence. Such chimeric peptides have been used very successfully to drive antibody production in mice, human/mice
5 chimeras and primates (Sharma et al., 1993; Ifversen et al., 1995; O'Hern et al., 1997).

In the present invention, the B cell epitope containing the first two to five amino acid residues of the free N-terminus or the last two to five amino acid residues of the
10 free C-terminus of an amyloid β peptide is fused, with or without spacer amino acid residues, to a known strong T helper cell epitope to form a chimeric peptide. A non-limiting example of such a known strong T cell epitope is the well-studied tetanus toxoid promiscuous epitope of SEQ ID NO:8 (Ho et al,
15 1990; Panina-Bordignon et al., 1989) as this epitope is known to work in a number of diverse human genetic backgrounds (Valmori et al., 1992 and 1994).

Immunization with the chimeric peptides(s) containing an amyloid β end-specific B cell epitope and a promiscuous T
20 helper cell epitope of tetanus toxoid, as a preferred embodiment, should give rise to antibodies with the following specificities:

(1) anti-tetanus antibody, which would be irrelevant in humans as most individuals are already sera-positive for
25 tetanus toxoid (i.e., from previous tetanus immunizations);

(2) anti-junction antibodies, which recognize novel epitopes created by the junction joining the end-specific B-cell epitope of an amyloid β peptide and a T helper cell epitope of tetanus toxoid, but would not recognize anything other than the
30 immunogen itself, and therefore would be irrelevant in vivo in the immunized individual; and

(3) anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies, which are the desired antibodies sought to be raised by the method according to the present invention for
35 inhibiting, reducing, or even perhaps reversing amyloid β deposit/plaque formation.

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The desired anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies raised by the method for immunization according to the present invention are able to discriminate between an amyloid β peptide and the β amyloid

5 protein precursor (βAPP) from which it is proteolytically derived. These end-specific amyloid β antibodies bind specifically to the terminus/end of an amyloid β peptide to slow down, reduce or prevent the accumulation of amyloid β peptides in the extracellular space, interstitial fluid and cerebrospinal

10 fluid of the brain, and the aggregation into senile amyloid deposits or plaques and to block the interaction of amyloid β peptides with other molecules that contribute to the neurotoxicity of amyloid β.

The presence of anti-N-terminal or anti-C-terminal

15 end-specific amyloid β antibodies in the blood and in the extracellular space, interstitial fluid and cerebrospinal fluid of the brain, where soluble amyloid β peptides are present, promotes the formation of soluble antibody-amyloid β complexes. These soluble antibody-amyloid β complexes are cleared from the

20 central nervous system by drainage of the extracellular space, interstitial fluid and cerebrospinal fluid into the general blood circulation through the arachnoid villi of the superior sagittal sinus. In this manner, soluble amyloid β peptides are prevented from accumulating in the extracellular space,

25 interstitial fluid and cerebrospinal fluid to form amyloid deposits and/or to induce neurotoxicity (Fig. 1). Furthermore, clearance of soluble amyloid-β peptides in accordance with the method of the present invention is expected to reduce the inflammatory process observed in Alzheimer's Disease by

30 inhibiting, for example, amyloid-β-induced complement activation and cytokine release, and by also blocking the interaction of Aβ with cell surface receptors such as the RAGE receptor.

As shown in Fig. 1 (see Schehr, 1994), and discussed in the Background Art section, the β-amyloid protein precursor

35 (βAPP) is believed to also serve as a precursor for a proteolytic product, soluble β-amyloid protein precursor (sβAPP), thought to have growth promoting and neuroprotective

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functions. It will be readily appreciated by those of skill in the art that the anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies will not interfere with the normal biological functions of βAPP that are not associated with the

5 formation of amyloid β peptides.

The advantages of the method for immunization according to the preferred embodiment of the present invention include:

(1) a cheap peptide antigen, that is readily and

10 easily produced and controlled for quality assurance, is used in active immunization;

(2) inclusion of only two to three, and perhaps up to four or five, amino acid residues from the N- or C- terminus of an internal peptide cleavage product (amyloid β peptide) should

15 minimize the amount of antibody produced which reacts with the precursor or mature protein from which the internal peptide cleavage product is proteolytically derived (βAPP), and hence might interfere with the function of this precursor or mature protein;

20   (3) use of an independent non-self T cell epitope should break self-tolerance and allow production of antibodies to a self-antigen (Schofield et al., 1991);

(4) the absence in the chimeric peptide(s) of a T cell epitope from the internal peptide cleavage product (amyloid β)

25 should avoid any significant problems of autoimmunity, since anti-self T cell immunity underlies progression of all known autoimmune diseases; and

(5) the immunization should be self-limiting and reversible, with antibody titers gradually falling off with

30 time, since the patient's immune system will never naturally encounter the combination of amyloid β with tetanus toxin as immunogen. This self-limiting and reversible immunization has been demonstrated in clinical trials with synthetic contraceptive vaccines (Talwar et al., 1997).

35   The chimeric peptide of the present invention is represented by formula (I) or formula (II)

(I) $N-(S)_m-(T_h)_n$

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(II) $(T_h)_n-(S)_m-C$
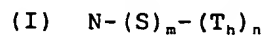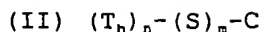
where:

N is the first 2, 3, 4 or 5 amino acid residues from the free N-terminus of a naturally-occurring internal peptide
5 cleavage product, such as an amyloid β peptide, which, when naturally-occurring in a mammal, is derived from a precursor protein or a mature protein;

C is the last 2, 3, 4 or 5 amino acid residues from the free C-terminus of the naturally occurring internal peptide
10 cleavage product;

T_h is a T helper cell epitope derived from a natural source (i.e., species of living organism) different from that of the naturally-occurring internal peptide cleavage product

S is a spacer amino acid residue;
15   m is 0, 1, 2, 3, 4, or 5; and

n is 1, 2, 3, or 4.

Also contemplated are mixtures of chimeric peptides of formula (I) and formula (II) in which the internal peptide cleavage product of each chimeric peptide can be the same or different,
20 i.e., different amyloid β peptides. These chimeric peptide mixtures are however not limited to mixtures of formula (I) and formula (II) chimeric peptides and can include mixtures of formula (I) peptides, where N is from different internal peptide cleavage products (i.e., different amyloid β peptides), or
25 mixtures of formula (II) peptides, where C is from different internal peptide cleavage products (i.e., different amyloid β peptides).

The chimeric peptide of the present invention has a length between about 20 to 90 amino acid residues, preferably
30 between about 20 to 75 amino acid residues, more preferably between about 20 to 50 amino acid residues, and most preferably between about 20 to 40 amino acid residues.

The B cell epitope from the free N-terminus (N) or the free C-terminus (C) of the naturally-occurring internal peptide
35 cleavage product is preferably two or three amino acid residues, which is not sufficient for raising antibodies cross-reactive with the precursor protein or the mature protein of the peptide

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cleavage product. A B cell epitope of four or five amino acid
residues from the free N-terminus or the free C-terminus of the
naturally-occurring internal peptide cleavage product may also
be suitable, although there is a slightly increased possibility
5 that some antibodies may be raised which are cross-reactive with
the precursor protein or the mature protein.

A preferred chimeric peptide embodiment of the present
invention has a N or C in formula (I) or (II), respectively,
from an amyloid β peptide which, when naturally-occurring in
10 humans, is derived from βAPP. As used herein, the term "derived
from" means that, when obtained from a natural source, the
peptide or protein is the result of cleavage or conversion from
a precursor protein or a mature protein, whether by a single
proteolytic cleavage event or by more than one cleavage and/or
15 conversion event. In the case of the preferred embodiment, any
amyloid β peptide found to occur naturally in amyloid deposits,
fibrils, and plaques is encompassed by the term "amyloid β
peptide" as the internal peptide cleavage product. Peptides of
SEQ ID NOs:2 (Aβ1-40), 3 (Aβ1-42), 4 (Aβ1-43), 5 (Aβ3-42), 6
20 (Aβ11-42), and 7 (Aβ17-42) are non-limiting examples of such
"amyloid β peptides".

T_h is a T helper cell epitope that is derived from a
natural source different from the source of the naturally-
occurring internal peptide cleavage product. In other words,
25 the T helper cell epitope is not recognized as part of a self-
molecule in the mammal subject immunized according to the method
of the present invention. The T helper cell epitope is combined
with a B cell epitope specific to the N-terminus or C-terminus
of peptide cleavage product which is internal (not located at
30 the ends) to a precursor or mature protein to evoke an efficient
antibody response.

It is known that immunogens must be presented in
conjunction with major histocompatibility (MHC) class II
antigens to evoke an efficient antibody response. The MHC class
35 II antigens produced by antigen-presenting cells (APCs) bind to
T cell epitopes present in the immunogen in a sequence specific
manner. This MHC class II-immunogen complex is recognized by

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CD4$^+$ lymphocytes (T$_h$ cells), which cause the proliferation of specific B cells capable of recognizing a B cell epitope from the presented immunogen and the production of B cell epitope-specific antibody by such B cells. Since amyloid β peptides are

5 self molecules, they do not possess any recognizable T$_h$ epitopes, and B cell epitopes of 2 to 5 amino acid residues would lack any T cell epitopes altogether. Such epitopes can be provided by specific sequences derived from potent immunogens including tetanus toxin, pertussis toxin, the measles virus F

10 protein and the hepatitis B virus surface antigen (HBsAg). The T$_h$ epitopes selected are preferably capable of eliciting T helper cell responses in large numbers of individuals expressing diverse MHC haplotypes. These epitopes function in many different individuals of a heterogeneous population and are

15 considered to be promiscuous T$_h$ epitopes. Promiscuous T$_h$ epitopes provide an advantage of eliciting potent antibody responses in most members of genetically diverse population groups.

Moreover, the T helper cell epitopes in the chimeric

20 peptide of the present invention are also advantageously selected not only for a capacity to cause immune responses in most members of a given population, but also for a capacity to cause memory/recall responses. When the mammal is human, the vast majority of human subjects/patients receiving immunotherapy

25 with the chimeric peptide of the present invention will already have been immunized with the pediatric vaccines (i.e., measles+mumps+rubella and diphtheria+pertussis+tetanus vaccines) and, possibly, the hepatitis B virus vaccine. These patients have therefore been previously exposed to at least one of the T$_h$

30 epitopes present in chimeric pediatric vaccines. Prior exposure to a T$_h$ epitope through immunization with the standard vaccines should establish T$_h$ cell clones which can immediately proliferate upon administration of the chimeric peptide (i.e., a recall response), thereby stimulating rapid B cell responses to

35 the chimeric peptide. In addition, the T$_h$ epitopes avoid any pathogen-specific B cell and/or suppressor T cell epitopes which could lead to carrier-induced immune suppression, a problem

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encountered when toxin molecules are used to elicit T helper
cell responses.

The T_h epitopes in the chimeric peptide of the
invention are promiscuous but not universal. This
5 characteristic means that the T_h epitopes are reactive in a
large segment of an outbred population expressing different MHC
antigens (reactive in 50 to 90% of the population), but not in
all members of that population. To provide a comprehensive,
approaching universal, immune reactivity for an internal peptide
10 cleavage product, a combination of chimeric peptides with
different T_h epitopes can be prepared. For example, a
combination of four chimeric peptides with promiscuous T_h
epitopes from tetanus and pertussis toxins, measles virus F
protein and HBsAg may be more effective.

15   Promiscuous T_h epitopes often share common structural
features. For example, promiscuous T_h epitopes range in size
from about 15 to about 30 residues. Amphipathic helices are a
common feature of the T_h epitopes. An amphipathic helix is
defined by an α-helical structure with hydrophobic amino acid
20 residues dominating the surrounding faces. T_h epitopes
frequently contain additional primary amino acid patterns such
as a Gly or a charged reside followed by two to three
hydrophobic residues followed in turn by a charged or polar
residue. This pattern defines Rothbard sequences. T_h epitopes
25 often obey the 1, 4, 5, 8 rule, where a positively charged
residue is followed by hydrophobic residues at the fourth, fifth
and eighth positions after the charged residue. Since all of
these structures are composed of common hydrophobic, charged and
polar amino acids, each structure can exist simultaneously
30 within a single T_h epitope.

T_h is therefore a sequence of amino acids (natural or
non-natural) that contain a T_h epitope. A T_h epitope can be a
continuous or discontinous epitope. Hence, not every amino acid
of T_h is necessarily part of the epitope. Accordingly, T_h
35 epitopes, including analogs and segments of T_h epitopes, are
capable of enhancing or stimulating an immune response to the
internal peptide cleavage product. Immunodominant T_h epitopes

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are broadly reactive in animal and human populations with widely divergent MHC types (Celis et al., 1988; Demotz et al., 1989; and Chong et al., 1992). The T_h domain of the chimeric peptides of the present invention has from about 10 to about 50 amino
5 acids residues and preferably from about 10 to about 30 amino acids residues. When multiple T_h epitopes are present (i.e., $n \geq 2$), then each T_h epitope is independently the same or different.

T_h epitope analogs include substitutions, deletions
10 and insertions of one to about five amino acid residues in the T_h epitope. T_h segments are contiguous portions of a T_h epitope that are sufficient to enhance or stimulate an immune response to the internal peptide cleavage product. An example of T_h segments is a series of overlapping peptides that are derived
15 from a single longer peptide.

The T_h epitopes of the present invention include hepatitis B surface antigen T helper cell epitopes (HB_s T_h), pertussis toxin T helper cell epitopes (PT T_h), tetanus toxin T helper cell epitopes (TT T_h), measles virus F protein T helper
20 cell epitope (MV_{F1} T_h), *Chlamydia trachomitis* major outer membrane protein T helper cell epitopes (CT T_h), diphtheria toxin T helper cell epitopes (DT T_h), *Plasmodium falciparum* circumsporozoite T helper cell epitopes (PF T_h), *Schistosoma mansoni* triose phosphate isomerase T helper cell epitopes (SM
25 T_h), *Escherichia coli* TraT T helper cell epitopes (TraT T_h) and immune-enhancing analogs and epitope sequences are provided below in Table 1.

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TABLE 1

TT_o T_h	SEQ ID NO:8
HB_3 T_h	SEQ ID NO:9
TT_1 T_h	SEQ ID NO:10
TT_1 T_h	SEQ ID NO:11
TT_2 T_h	SEQ ID NO:12
PT_{1A} T_h	SEQ ID NO:13
TT_3 T_h	SEQ ID NO:14
PT_2 T_h	SEQ ID NO:15
MV_{F1} T_h	SEQ ID NO:16
MV_{F2} T_h	SEQ ID NO:17
TT_4 T_h	SEQ ID NO:18
TT_5 T_h	SEQ ID NO:19
CT_1 T_h	SEQ ID NO:20
DT_1 T_h	SEQ ID NO:21
DT_2 T_h	SEQ ID NO:22
PF T_h	SEQ ID NO:23
SM T_h	SEQ ID NO:24
$TraT_1$ T_h	SEQ ID NO:25
$TraT_2$ T_h	SEQ ID NO:26
$TraT_3$ T_h	SEQ ID NO:27

Immunogenicity can be improved through the addition of spacer residue(s) S (i.e. Gly-Gly) between the promiscuous T_h epitope and the B cell epitope of the chimeric peptide according to the present invention. In addition to physically separating the T_h epitope from the B cell epitope, the glycine spacer residues can disrupt any artificial secondary structures created by the joining of the T_h epitope with the B cell epitope, and thereby eliminate interference between the T and/or B cell responses. The conformational separation between the helper epitope and the antibody eliciting domain thus permits more efficient interactions between the presented immunogen and the appropriate T_h and B cells. The amino acid residue(s) for S can

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be naturally-occurring amino acids or non-naturally-occurring
amino acids, which include, but are not limited to, β-alanine,
ornithine, norleucine, norvaline, hydroxyproline, thyroxine,
gamma-amino butyric acid, homoserine, citrulline and the like.

5   The chimeric peptides of the present invention can be
made by synthetic chemical methods which are well known to the
ordinarily skilled artisan. Hence, the chimeric peptides can be
synthesized using the automated Merrifield techniques of solid
phase synthesis with either t-Boc or F-moc chemistry on Peptide
10 Synthesizers such as an Applied Biosystems Peptide Synthesizer.
          After complete assembly of the desired chimeric
peptide, the resin is treated according to standard procedures
to cleave the peptide from the resin and deblock the protecting
groups on the amino acid side chains. The free peptide is
15 purified by HPLC and characterized biochemically, for example,
by amino acid analysis or by sequencing. Purification and
characterization methods for peptides are well-known to one of
ordinary skill in the art.
          Alternatively, the longer linear chimeric peptides can
20 be synthesized by well-known recombinant DNA techniques. Any
standard manual on DNA technology provides detailed protocols to
produce the chimeric peptides of the invention. To construct a
gene encoding a chimeric peptide of the present invention, the
amino acid sequence is reverse transcribed into a nucleic acid
25 sequence, and preferably using optimized codon usage for the
organism in which the gene will be expressed. Next, a synthetic
gene is made, typically by synthesizing overlapping
oligonucleotides which encode the peptide and any regulatory
elements, if necessary. The synthetic gene is inserted in a
30 suitable cloning vector and recombinant clones are obtained and
characterized. The chimeric peptide is then expressed under
suitable conditions appropriate for the selected expression
system and host, and the chimeric peptide is purified and
characterized by standard methods.
35   As an optional segment to the chimeric peptide, an
immunostimulatory epitope of the invasin protein of a *Yersinia*
species can be linked to the T helper cell epitope of the

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chimeric peptide opposite from the B cell epitope. The invasins
of the pathogenic bacteria Yersinia spp. are outer membrane
proteins which mediate entry of the bacteria into mammalian
cells (Isberg et al., 1990). Invasins of cultured mammalian
5 cells by the bacterium was demonstrated to require interaction
between the Yersinia invasin molecule and several species of the
β1 family of integrins present on the cultured cells (Tran Van
Nhieu et al., 1991) Since T lymphocytes are rich in β1 integrins
(especially activated immune or memory T cells) the effects of
10 invasin on human T cell have been investigated (Brett et al.,
1993). It is thought that integrins facilitate the migration of
immune T cells out of the blood vessels and through connective
tissues to sites of antigenic challenge through their
interaction with extracellular matrix proteins including
15 fibronectin, laminin and collagen. The carboxy-terminus of the
invasin molecule was found to be co-stimulatory for naive human
CD4$^+$ T in the presence of the non-specific mitogen, anti-CD3
antibody, causing marked proliferation and expression of
cytokines. The specific invasin domain which interacts with the
20 β1 integrins to cause this stimulation also was identified
(Brett et al., 1993). Because of the demonstrated T cell co-
stimulatory properties associated with this domain, it can be
linked to the promiscuous T_h epitope in the chimeric peptide of
the present invention opposite from the B cell epitope.
25   Many of the outer membrane proteins of Gram-negative
bacteria are both lipid-modified and very immunogenic. Because
of the apparent correlation between covalent lipid linkage and
immunogenicity, tripalmitoyl-S-glycerol cysteine (Pam$_3$Cys), a
lipid common to bacterial membrane proteins, can be coupled to
30 synthetic peptides representing either B cell or cytotoxic T
cell epitopes. Because significant adjuvanting responses are
elicited by this lipid linkage, promiscuous T_h epitope of the
chimeric peptide can be lipid modified opposite its linkage to
the B cell epitope. Such lipid-modified chimeric peptides are
35 likely to be more immunogenic than the unmodified version of the
same peptide.

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U.S. Patent 5,843,446, which includes a disclosure of T_h epitopes and the immunostimulatory properties of invasin epitopes and lipid moieties, is herein incorporated entirely by reference.

5   Immunogenicity can further be significantly improved if the antigens are co-administered with adjuvants. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a

10 depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

          Immunostimulatory agents or adjuvants have been used

15 for many years to improve the host immune responses, e.g. to vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are

20 formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed,

25 only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diptheria and tetanus toxoids is well established and a HBsAg vaccine has been adjuvanted with

30 alum as well.

          A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in

35 mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes. To efficiently induce humoral

immune responses (HIR) and cell-mediated immunity (CMI),
immunogens are emulsified in adjuvants. Many adjuvants are
toxic, inducing granulomas, acute and chronic inflammations
(Freund's complete adjuvant, FCA), cytolysis (saponins and
5 Pluronic polymers) and pyrogenicity, arthritis and anterior
uveitis (LPS and MDP). Although FCA is an excellent adjuvant
and widely used in research, it is not licensed for use in human
or veterinary vaccines because of its toxicity.

         U.S. Pat. No. 4,855,283 teaches glycolipid analogues
10 including N-glycosylamides, N-glycosylureas and N-
glycosylcarbamates, each of which is substituted in the sugar
residue by an amino acid, as immuno-modulators or adjuvants.
U.S. Pat. No. 4,258,029 teaches that octadecyl tyrosine
hydrochloride (OTH) functions as an adjuvant when complexed with
15 tetanus toxoid and formalin inactived type I, II and III
poliomyelitis virus vaccine. Also, Nixon-George et al., 1990,
reported that octadecyl esters of aromatic amino acids complexed
with a recombinant hepatitis B surface antigen enhanced the host
immune responses against hepatitis B virus.

20   The addition of exogenous adjuvant/emulsion
formulations which maximize immune responses to the internal
peptide cleavage product are preferred. The adjuvants and
carriers that are suitable are those: (1) which have been
successfully used in Phase I human trials; (2) based upon their
25 lack of reactogenicity in preclinical safety studies, have
potential for approval for use in humans; or (3) have been
approved for use in food and companion animals.

         Immunotherapy regimens which produce maximal immune
responses following the administration of the fewest number of
30 doses, ideally only one dose, are highly desirable. This result
can be approached through entrapment of immunogen in
microparticles. For example, the absorbable suture material
poly(lactide-co-glycolide) co-polymer can be fashioned into
microparticles containing immunogen. Following oral or
35 parenteral administration, microparticle hydrolysis *in vivo*
produces the non-toxic byproducts, lactic and glycolic acids,
and releases immunogen largely unaltered by the entrapment

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process. The rate of microparticle degradation and the release
of entrapped immunogen can be controlled by several parameters,
which include (1) the ratio of polymers used in particle
formation (particles with higher co-glycolide concentrations

5 degrade more rapidly); (2) particle size, (smaller particles
degrade more rapidly than larger ones); and, (3) entrapment
efficiency, (particles with higher concentrations of entrapped
antigen degrade more rapidly than particle with lower loads).
Microparticle formulations can also provide primary and

10 subsequent booster immunizations in a single administration by
mixing immunogen entrapped microparticles with different release
rates. Single dose formulations capable of releasing antigen
ranging from less than one week to greater than six months can
be readily achieved. Moreover, delivery of the chimeric peptide

15 according to the present invention entrapped in microparticles
can also provide improved efficacy when the microparticulate
immunogen is mixed with an exogenous adjuvant/emulsion
formulations.

        The efficacy of the chimeric peptides can be

20 established and analyzed by injecting an animal, e.g., mice or
rats, with the chimeric peptide formulated in alum and then
following the immune response to the internal peptide cleavage
product.

        Another aspect of the present invention provides an

25 immunizing composition which includes an immunizing effective
amount of one or more of the chimeric peptides of the invention
and a pharmaceutically acceptable carrier, excipient, diluent,
or auxiliary agent, including adjuvants. Accordingly, the
chimeric peptides can be formulated as an immunizing composition

30 using adjuvants, pharmaceutically-acceptable carriers,
excipients, diluents, auxiliary agents or other ingredients
routinely provided in immunizing compositions. Such
formulations are readily determined by one of ordinary skill in
the art and include formulations for immediate release and for

35 sustained release, e.g., microencapsulation. The present
immunizing compositions can be administered by any convenient
route including subcutaneous, oral, intramuscular, or other

parenteral or internal route. Similarly the vaccines can be
administered as a single dose or divided into multiple doses for
administration. Immunization schedules are readily determined
by the ordinary skilled artisan. For example, the adjuvants or
5 emulsifiers that can be used in this invention include alum,
incomplete Freund's adjuvant, liposyn, saponin, squalene, L121,
emulsigen and ISA720. In preferred embodiments, the
adjuvants/emulsifiers are alum, incomplete Freund's adjuvant, a
combination of liposyn and saponin, a combination of squalene
10 and L121 or a combination of emulsigned and saponin.

The immunizing compositions of the present invention
contain an immunoeffective amount of one or more of the chimeric
peptides and a pharmaceutically acceptable carrier. Such
compositions in dosage unit form can contain about 0.5 µg to
15 about 1 mg of each peptide per kg body weight. When delivered
in multiple doses, the dosage unit form is conveniently divided
into the appropriate amounts per dosage.

Immunizing compositions which contain cocktails of two
or more of the chimeric peptides of the present invention
20 enhance immunoefficacy in a broader population and thus provide
a better immune response against the internal peptide cleavage
product, such as amyloid β. Other immunostimulatory synthetic
chimeric peptide immunogens are arrived at through modification
into lipopeptides so as to provide built-in adjuvanticity for
25 potent vaccines. The immune response to synthetic chimeric
peptide immunogens of the present invention can be improved by
delivery through entrapment in or on biodegradable
microparticles of the type described by O'Hagan et al (1991).
The immunogens can be encapsulated with or without adjuvant,
30 including covalently attached lipid moiety such as Pam_3Cys, and
such microparticles can be administered with an
immunostimulatory adjuvant such as Freund's Incomplete Adjuvant
or alum. The microparticles function to potentiate immune
responses to an immunogen and to provide time-controlled release
35 for sustained or periodic responses. for oral administration,
and for topical administration (O'Hagan et al., 1991).

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A further aspect of the present invention is a method for immunization against the free N-terminus and/or free C-terminus of a naturally-occurring internal peptide cleavage product, which, when naturally-occurring in a mammal, is derived
5 from a precursor protein or a mature protein. This method according to the present invention involves administering to a mammal an immunizing composition containing the chimeric peptides of the present invention for which the internal peptide cleavage product is a self molecule. A preferred embodiment of
10 this method is one in which the method is directed to immunizing against amyloid β peptides to raise anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies which do not cross-react with the β amyloid precursor protein. In this preferred embodiment, the N and/or C of formulas (I) and (II) of the
15 chimeric peptide(s) used in the present method are from the N-terminus and/or C-terminus of amyloid β peptides.

A still further aspect of the present invention is a molecule which contains the antigen-binding portion of an antibody, preferably a monoclonal antibody, specific for the
20 chimeric peptide according to the present invention. This molecule can be an antibody raised against the chimeric peptide *in vivo* or can be a recombinant antibody that is intended to encompass fragments of antibodies containing the antigen-binding portion, chimeric or humanized immunoglobulin molecules of any
25 isotype, as well as a single-chain antibodies.

Chimeric antibodies are understood to be molecules, different portions of which are derived from different animal species, such as those humanized antibodies having a variable region derived from a mouse monoclonal antibody and a human
30 immunoglobulin constant region. Chimeric antibodies and methods for their production are well known in the art. For example, the DNA encoding the variable region of the antibody can be inserted into or joined with DNA encoding other antibodies to produce chimeric antibodies (U.S. patent 4,816,567; Orlandi et
35 al., 1989).

Single-chain antibodies can be single chain composite polypeptides having end-specific peptide binding capability and

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comprising a pair of amino acid sequences homologous or
analogous to the variable regions of an immunoglobulin light and
heavy chain (linked V_H-V_L or single chain Fv). Both V_H and V_L
may copy natural monoclonal antibody sequences, or one or both
5   of the chains may comprise a CDR-FR construct of the type
described in U.S. Patent 5,091,513. The separate polypeptides
analogous to the variable regions of the light and heavy chains
are held together by a peptide linker. Methods of production of
such single chain antibodies, e.g., single chain Fv (scFv),
10 particularly where the DNA encoding the polypeptide structures
of the V_H and V_L chains are characterized or can be readily
ascertained by sequence analysis, may be accomplished in
accordance with the methods described, for example, in U.S.
Patent 4,946,778, U.S. Patent 5,091,513, U.S. Patent 5,096,815,
15 Biocca et al. (1993), Duan et al. (1994), Mhashilkar et al.
(1995), Marasco et al. (1993), and Richardson et al. (1995).

        Besides the conventional method of raising antibodies
in vivo, antibodies can be produced *in vitro* using Phage Display
technology. The production of recombinant antibodies is much
20 faster compared to conventional antibody production and they can
be generated against an enormous number of antigens. In
contrast, in the conventional method, many antigens prove to be
non-immunogenic or extremely toxic, and therefore cannot be used
to generate antibodies in animals. Moreover, affinity
25 maturation (i.e., increasing the affinity and specificity) of
recombinant antibodies is very simple and relatively fast.
Finally, large numbers of different antibodies against a
specific antigen can be generated in one selection procedure.
To generate recombinant monoclonal antibodies one can use
30 various methods all based on Phage Display libraries to generate
a large pool of antibodies with different antigen recognition
sites. Such a library can be made in several ways: One can
generate a synthetic repertoire by cloning synthetic CDR3
regions in a pool of heavy chain germline genes and thus
35 generating a large antibody repertoire, from which recombinant
antibody fragments with various specificities can be selected.
One can use the lymphocyte pool of humans as starting material

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for the construction of an antibody library. It is possible to
construct naive repertoires of human IgM antibodies and thus
create a human library of large diversity. This method has been
widely used successfully to select a large number of antibodies
5 against different antigens.

Yet another aspect of the present invention is a
method for passive immunization involving administering to a
mammal, preferably human, the molecule which contains the
antigen-binding portion of an antibody specific for the chimeric
10 peptide according to the present invention for which the
internal peptide cleavage product is a self molecule. A
preferred embodiment of this method is one in which the method
is directed to passively immunizing with molecules in which the
antigen-binding portion thereof is end-specific for amyloid β
15 peptides.

Having now fully described this invention, it will be
appreciated by those skilled in the art that the same can be
performed within a wide range of equivalent parameters,
concentrations, and conditions without departing from the spirit
20 and scope of the invention and without undue experimentation.

While this invention has been described in connection
with specific embodiments thereof, it will be understood that it
is capable of further modifications. This application is
intended to cover any variations, uses, or adaptations of the
25 inventions following, in general, the principles of the
invention and including such departures from the present
disclosure as come within known or customary practice within the
art to which the invention pertains and as may be applied to the
essential features hereinbefore set forth as follows in the
30 scope of the appended claims.

All references cited herein, including journal
articles or abstracts, published or corresponding U.S. or
foreign patent applications, issued U.S. or foreign patents, or
any other references, are entirely incorporated by reference
35 herein, including all data, tables, figures, and text presented
in the cited references. Additionally, the entire contents of

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the references cited within the references cited herein are also entirely incorporated by references.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way
5 an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that
10 others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore,
15 such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the
20 terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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- 34 -

WHAT IS CLAIMED IS:

1. A chimeric peptide represented by formula (I) or formula (II),

(I) $N-(S)_m-(T_h)_n$

5   (II) $(T_h)_n-(S)_m-C$
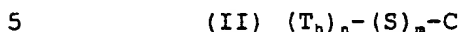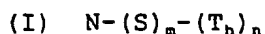
or chimeric peptides which are mixtures of formula (I) peptides, mixtures of formula (II) peptides, or mixtures of formula (I) and formula (II) peptides, wherein:

N is the first 2, 3, 4, or 5 amino acid residues from
10 the free N-terminus of a naturally-occurring internal peptide cleavage product which, when naturally-occurring in a mammal, is derived from a precursor protein or a mature protein;

C is the last 2, 3, 4, or 5 amino acid residues from the free C-terminus of said naturally-occurring internal peptide
15 cleavage product;

T_h is a T helper cell epitope;

S is a spacer amino acid residue;

m is 0, 1, 2, 3, 4, or 5; and

n is 1, 2, 3, or 4.

20   2. The chimeric peptide or peptides according to claim 1, wherein said internal peptide cleavage product is an amyloid

β peptide, which, when naturally-occurring, is derived from cleavage of β amyloid precursor protein (βAPP).

25   3. The chimeric peptide or peptides according to claim 2, wherein said internal peptide cleavage product has an amino acid sequence selected from the group consisting of SEQ ID NOs:2, 3, 4, 5, 6, 7, and mixtures thereof.

4. The chimeric peptide or peptides according to
30 claim 1, wherein N is the first 2 or 3 amino acid residues from the free N-terminus of said internal peptide cleavage product.

5. The chimeric peptide or peptides according to claim 1, wherein C is the last 2 or 3 amino acid residues from the free C-terminus of said internal peptide cleavage product.

35   6. The chimeric peptide or peptides according to claim 1, wherein T_h is a promiscuous T helper cell epitope.

7.   The chimeric peptide or peptides according to
claim 6, wherein said promiscuous T helper cell epitope is
derived from tetanus toxin, pertussis toxin, diphtheria toxin,
measles virus F protein, hepatitis B virus surface antigen,
5 *Chlamydia trachomitis* major outer membrane protein, *Plasmodium
falciparum* circumsporozoite, *Schistosoma mansoni* triose
phosphate isomerase, or *Escherichia coli* TraT.

8.   The chimeric peptide or peptides according to
claim 7, wherein said promiscuous T helper cell epitope has an
10 amino acid sequence selected from the group consisting of SEQ ID
NOs:8 to 27.

9.   The chimeric peptide or peptides according to
claim 1, wherein S is glycine.

10.   An immunizing composition, comprising an
15 immunizing effective amount of the chimeric peptide or peptides
according to claim 1 and a pharmaceutically acceptable carrier,
excipient, diluent, or auxiliary agent.

11.   The immunizing composition according to claim 10,
wherein said pharmaceutically acceptable auxiliary agent is an
20 adjuvant.

12.   The immunizing composition according to claim 11,
wherein said adjuvant is alum.

13.   A method for immunization against the free N-
terminus or free C-terminus of an internal self peptide cleavage
25 product derived from a precursor protein or a mature protein,
comprising administering to a mammal the immunizing composition
according to claim 10, for which the internal peptide cleavage
product is a self molecule of the mammal.

14.   The method according to claim 13, wherein the
30 mammal is a human.

15.   The method according to claim 14, wherein the
internal self peptide cleavage product is an amyloid β peptide,
which when naturally-occurring, is derived from cleavage of β
amyloid precursor protein, whereby said method raises antibodies
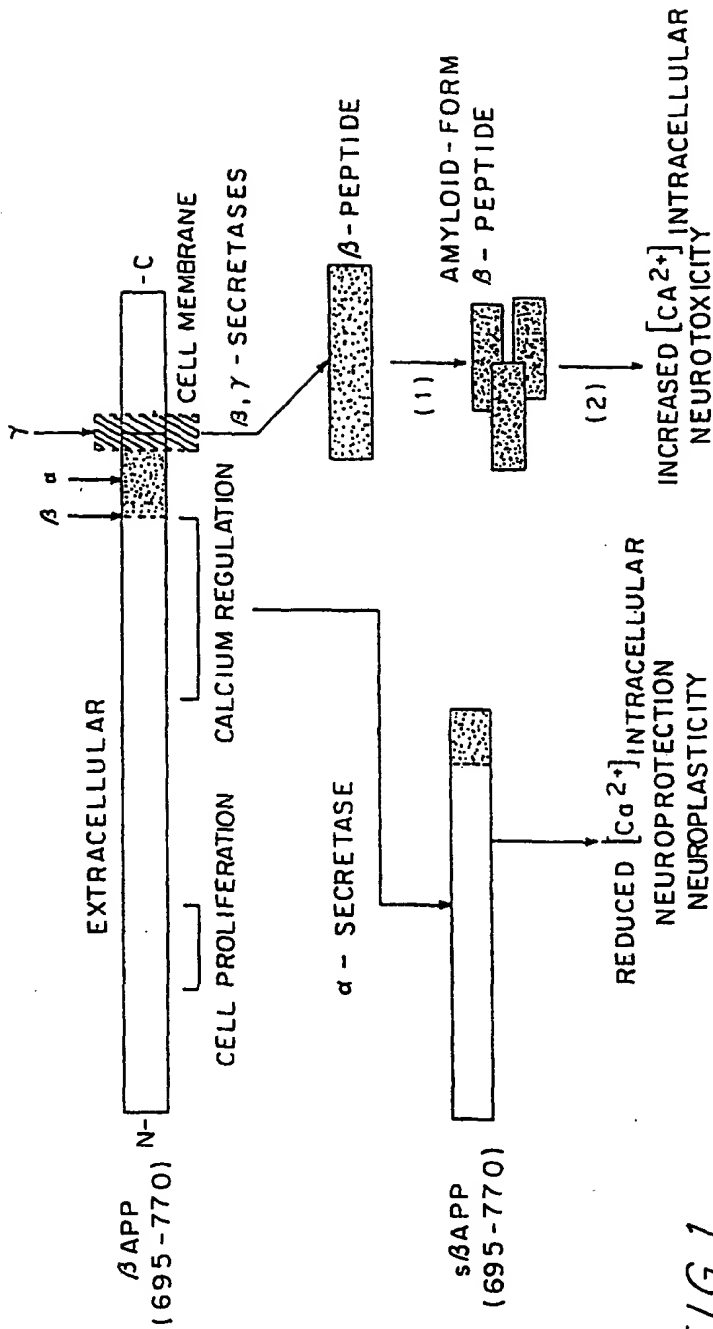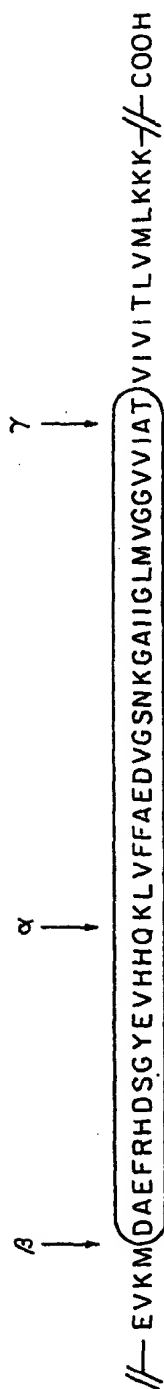35 specific to the free N-terminus and/or free C-terminus of the
amyloid β peptide.

FIG. 1

FIG. 2

//— EVKM (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT) VIVITLVMLKKK —//— COOH

β →   α →   γ →

SEQUENCE LISTING

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IMMUNIZATION USING CHIMERIC PEPTIDES OR ANTIBODIES

<130> CHAIN8A

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<141> 2000-12-08

<150> 60/169,687
<151> 1999-12-08

<160> 27

<170> PatentIn version 3.0

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Thr

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CHIMERIC PEPTIDES (SHORT B-CELL EPITOPE JOINED TO A T-CELL EPITOPE) AND THEIR USE FOR IMMUNIZATION

//— EVKM (DAEFRHDSG YEVHHQ KLVFFAEDVGSNKGAIIGLMVGGVVIAT) VIVI TLVMLKKK —//— COOH

(57) Abstract: The invention provides a chimeric peptide or mixture of chimeric peptides that can be formulated as an immunizing composition and used in a method for immunization of a mammal against an internal peptide cleavage product derived from a precursor or mature protein, for which the peptide cleavage product and the precursor or mature protein are self molecules. The chimeric peptide or peptides have an end-specific B cell epitope, of 2-5 amino acids, from a naturally-occurring internal peptide cleavage product of a precursor or mature protein, as a free N- or C- terminus, fused with or without spacer residues to a T helper cell epitope derived from a living source different from that of the internal peptide cleavage product.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K19/00 C07K14/47 A61K38/16 A61K39/00 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FRENKEL ET AL: "N-terminal EFRH sequence of Alzheimer's beta-amyloid peptide represents the epitope of its anti-aggregating antibodies" JOURNAL OF NEUROIMMUNOLOGY, vol. 88, 1998, pages 85-90, XP001001343 * Pages 89-90 (Discussion) *	1-20

-/--

☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 2001	11/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl. Fax: (+31–70) 340–3016	Korsner, S-E

Form PCT/ISA/210 (second sheet) (July 1992)

International plication No

PCT/US 00/33203

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document. with indication,where appropriate. of the relevant passages	Relevant to claim No.
Y	DELMAS A ET AL: "The binding of chimeric peptides to GM1 ganglioside enables induction of antibody responses after intranasal immunization" VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 14, no. 11, 1 August 1996 (1996-08-01), pages 1077-1082, XP004057643 ISSN: 0264-410X * Page 1078 (Table 1); pages 1080-81 (Discussion) *	1-20
Y	PARTIDOS C ET AL: "The influence of orientation and number of copies of T and B cell epitopes on the specificity and affinity of antibodies induced by chimeric peptides" EUROPEAN JOURNAL OF IMMUNOLOGY,DE,WEINHEIM, vol. 22, no. 10, 1 October 1992 (1992-10-01), pages 2675-2680, XP000607498 ISSN: 0014-2980 * Page 2676 (Table 1); page 2679 (column 2) *	1-20
Y	O'HERN P A ET AL: "Colinear synthesis of an antigen-specific B-cell epitope with promiscuous tetanus toxin T-cell epitope: a synthetic peptide immunocontraceptive" VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 15, no. 16, 1 November 1997 (1997-11-01), pages 1761-1766, XP004091952 ISSN: 0264-410X * Figure 1 (tetanus toxin) *	1-20
A	BRONS N H C ET AL: "Hierarchic T-cell help to non-linked B-cell epitopes" SCANDINAVIAN JOURNAL OF IMMUNOLOGY,GB,OXFORD, vol. 44, no. 5, 1996, pages 478-484, XP000910022 ISSN: 0300-9477 * Pages 282-284 (Discussion) *	1-20
Y	WO 99 27944 A (ATHENA NEUROSCIENCE) 10 June 1999 (1999-06-10) * Pages 13-15 (peptides), 19-20 (tetanus toxoid), 49 (DAEFR) *	1-20

-/-

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 72880 A (NEURALAB LIMITED) 7 December 2000 (2000-12-07) * Pages 4-6 (peptides), 14-15 (peptides), 28 (universal T-cell epitopes), 61 (DAEFR, line 2), 100 (Table 18) *	1-20

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.:   1-20 (partially)

Present claims 1-20 relate to an extremely large number of possible and UNDEFINED peptides.
Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds;   namely the peptides where the B-cell epitope is the first (last) 2-5 amino acids from N- (or C-terminal) of the cleavage product of the AMYLOID PRECURSOR PROTEIN.

Consequently, the international search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds with amyloid fragments as defined above.

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The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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